IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Akira Ito et al.
Serial No.: 10/815,273
Filed: March 31, 2004
For: HYPERTHERMIA AGENT FOR MALIGNANT TUMOR COMPRISING CYTOKINE AND MAGNETIC FINE PARTICLES
Art Unit: 1642
Examiner: Laura B. Goddard, Ph.D.

DECLARATION UNDER 37 CFR 1.132

I. I, Takeshi Kobayashi, an inventor of this case, declare and say as follows.

I am one of the joint inventors of the U.S. Patent Application as identified above and understand the English language. I studied the Official Action dated November 1, 2005 received in the above-identified application and have reviewed the documents cited by the Official Action of November 1, 2005.

The Official Action of November 1, 2005 cited the following two documents:

Cited document 1: Jpn J Cancer Research, 1998, 89:463-470 (Yanase et al.), which discloses hyperthermia.

Cited document 2: Clinical Cancer Research, 2002, 8:2775-2781 (O' Day et al.), which pertains to use of interleukin-2 ("IL-2") and granulocyte macrophage-colony stimulating factor ("GM-CSF") treatment.

Comparison with Cited document 1 (hyperthermia treatment)

In Cited document 1, using magnetite encapsulated in a cationic liposome for hyperthermia has been disclosed. Rats were used as experimental animals, at the 11th day from transplantation of T-9 cells, magnetite (amount of the magnetite: 3 mg, Volume: 0.4 ml) was injected into the tumor, and after 24 hours from the injection, an initial irradiation was carried out. Moreover, additional irradiations were carried out after 24 hours and 48 hours from the initial irradiation. Each irradiation time was 30 minutes. A group of rats which was irradiated once showed 20% CR (complete-regression, hereinafter "CR"), a group of rats that was irradiated twice showed 60% CR and a group of rats that was irradiated three times showed 87% CR, as shown in Table I on page 465 of the cited reference.

In the above-identified application, mice were used as experimental animals. After transplantation of mouse melanoma B16 cells, magnetite (amount of the magnetite: 2 mg, Volume: 0.1 ml) was injected into the tumor after the tumor had grown to a diameter of 6 mm, and immediately after the injection, irradiation was carried out once for 30 minutes.

An average tumor volume of the mice, as described in Example 1 (and Figure 5) on pages 9-13 of the specification of the above-identified application ("the Specification"), was determined. After the 15th day from the administration of the magnetite, as disclosed in Fig. 5 of the Specification, the control group had a tumor average volume of about 2900 mm^3, and the irradiated group (no cytokine) had a tumor average volume of about 1000 mm^3 (which is 34% of the average volume of the control group). Thus, the irradiation helped reduce the growth of the tumor. However, 7 of 10 of the mice which were irradiated died by around the 25th day after the injection of the magnetite and the tumor

growth of the three remaining mice was uncontrolled. Also, CR of the once irradiated group in this experiment was 0%.

In sum, the CR after one irradiation in document 1 was 20% and the CR after one irradiation as described in the Specification was 0% with the hyperthermia treatment only.

Comparison with Cited document 2 (IL-2 and GM-CSF treatment)

Cited document 2 discloses a therapeutic method which combines the following three steps and is carried out in a Phase 2 test with regard to metastatic melanoma.

Step 1) Maintaining therapy of IL-2: 1 cycle (20 MIU/m^2 in total seems to be injected) for 28 days from Monday to Friday,

Step 2) Pulse therapy of IL-2: 18 MIU/m^2 for 6 hours → 18 MIU/m^2 for 12 hours → 18 MIU/m^2 for 24 hours (54 MIU/m^2 in total seems to be injected) over Saturday and Sunday,

Step 3) Maintaining therapy of GM-CSF: in 1 cycle, 125 μg/m^2 was administered for 14 days continuously.

For each patient, 4 cycles (28 days for 1 cycle) in average were carried out, and the results are 15% in CR and 12% in SD (Stable disease) (as shown in document 2 on p. 2777, right column, lines 45-47, and in Table 3).

In the above-identified application, the administrations of IL-2 and GM-CSF are mutually exclusive. In contrast, in document 2 they are administered together. The amount of the administration in the above-identified application is 1×10^5 U of IL-2, and 20 μg of GM-CSF in total.

In Example 1 of the Specification, IL-2 testing is carried out. An average tumor volume of the mice as described in Example 1 on pages 9-13 of the Specification was measured. On the 15th day from the

administration of the magnetite, as seen in Figure 5, the control demonstrated a tumor average volume of about 2900 mm^3, and the group treated with IL-2 without irradiation demonstrated a tumor average volume of about 650 mm^3 (which is 22% of the average volume of the control group). Thus, in the IL-2 treated group without irradiation in Example 1 of the Specification, there was some inhibition of tumor growth. However, one of the mice died after about 20 days. Of the 7 mice that lived, 5 of the 7 had uncontrolled growth. Thus, of the living mice 71.4% of them had uncontrolled growth. Of the 8 mice, only 1 has what appears to be a decreasing tumor size and one appears to have a tumor size that has remained constant. Thus, the CR is at most 12.5% since one mouse appeared to have a decreasing tumor size.

Example 2, mentioned on pages 14-18 of the Specification, discloses the administration of GM-CSF (no irradiation) and gave substantially the same results as with the administration of IL-2 (Fig. 9 and Fig. 11). In the group where GM-CSF was administered without irradiation, all of the mice appeared to have increasing tumor size. Thus, the CR is 0% as can be seen from Figure 9C.

In sum, CR in document 2 was 15% for a combination of IL-2 and GM-CSF treatment. In the above-identified application, the CR for the non-irradiated group that had been treated with IL-2 is 12.5% at most. In the above-identified application, the CR for the non-irradiated group that had been treated with GM-CSF is 0%.

Comparison with irradiation and IL-2 or irradiation and GM-CSF

An average tumor volume of the mice as treated according to Example 1 (hyperthermia and IL-2 treatment), as described on pages 9-13 of the Specification, was measured. On the 15th day from the

administration of the magnetite, as can be seen from Figure 5 of the Specification, the control group had a tumor average size of about 2900 mm^3, and the group in which hyperthermia and IL-2 were used in combination is about 20 mm^3 (which is 0.7% of the average tumor size for the control). The group that was subjected to hyperthermia but which had no IL-2 added demonstrated after 15 days a tumor average size of about 1000 mm^3 (which is 34 % as that of the control), and the group that was treated with IL-2 but not subjected to hyperthermia showed a 15-day tumor average size of about 650 mm^3 (which is 22% as that of the control). Thus, it is evident that the use of both hyperthermia and IL-2 provides significant advantages over the use of only hyperthermia or only IL-2. In fact, the effects of both hyperthermia and IL-2 are synergistic and unexpected based on the results of the hyperthermia or the IL-2 independently.

Furthermore, of the 8 mice that were treated by both hyperthermia and IL-2, 6 out of the 8 saw a complete regression of the tumor (although one mouse had a recurrence of the tumor after it had been completely regressed). Thus, the CR is 75%. In contrast, the CR for the group treated with hyperthermia only was 0% and the CR for the group treated with IL-2 only was 12.5%. Thus, it is clear that the CR provided by the combination of hyperthermia and IL-2 provides significant advantages over the use of only hyperthermia or only IL-2. In fact, the effects of both hyperthermia and IL-2 are synergistic and unexpected based on the results of the hyperthermia or the IL-2 independently.

Even if the CR of 20% disclosed in the cited document 1 for one irradiation is used instead of the results of the Specification (which was 0% CR), it is still evident that the effects of both hyperthermia and IL-2 are synergistic and therefore unexpected based on the results of the hyperthermia or the IL-2 independently.

Additionally, in the control group, in the group that had hyperthermia treatment only, and in the group that had IL-2 treatment only, all the mice died after 80 days. In contrast, the group that utilized hyperthermia and IL-2 had a survival rate of 5 out of 8 mice (62.5%). Thus, it can be seen that the use of both hyperthermia and IL-2 to treat a tumor has advantageous, synergistic, and unexpected effects.

In Example 2 (GM-CSF treatment) mentioned on pages 14-18 of the Specification, 7 of 10 mice died by the 30^{th} day after administration of the magnetite in the control group. In the group treated by only irradiation and in the group treated only by GM-CSF, 7 of 10 mice died by the 30^{th} day after administration of the magnetite. In contrast, the group of mice subjected to hyperthermia and GM-CSF had four mice (out of ten) where the tumor disappeared. This is a CR of 40%. No regression of the tumor was observed. Also, in the control group, in the group with only hyperthermia treatment, and in the group with only GM-CSF treatment, all the mice died by the 50^{th} day after administration of the magnetite. In contrast, the group that was subject to hyperthermia and GM-CSF had a survival rate of 50% after 50 days.

According to the above, by using a combination of hyperthermia and cytokine as described in the Specification, excellent anti-tumor effect and extension of life is seen in a synergistic manner. Thus, it is clear that the methodology disclosed in the Specification of a combined therapy of hyperthermia and cytokine for the treatment of tumors shows an unexpectedly high anti-tumor effect when the data of the Specification is analyzed and even when the data of the cited documents 1 and 2 is also used for comparison. Moreover, it should also be noted that in the cited document 2, there is a combination of IL-2 and GM-CSF that is used, as opposed to each individually. As the cited document 2 states on page 1, column 2, the effect of the IL-2 and GM-CSF combination is better than the

effect of each separately. Even if the numbers for cited document 2 are compared with the invention of the above-identified application, it is still clear that the use of hyperthermia and cytokine is synergistically better than used separately.

III. Conclusion

In sum, the following results are summarized:

Cited document 1: Hyperthermia treatment only. Complete regression ("CR") was observed in 20% of rats after a total of one irradiation.

Cited document 2:Treatment consisted if IL-2 and GM-CSF. CR for this treatment was 15%. This 15% would have been lower if IL-2 or GM-CSF had been provided separately, as disclosed in the document itself.

Example 1 of above-identified application: Treatment included control, hyperthermia treatment only, IL-2 treatment only, and both hyperthermia and IL-2 treatment. Hyperthermia-only treatment (one irradiation) helped to reduce tumor growth to 34% as opposed to no hyperthermia, when measured after 15 days from injection of magnetite. The CR of the group treated with hyperthermia only was 0%. Seven of the ten mice died within 30 days and the three had uncontrolled growth of tumors in the hyperthermia-only treatment group. All the mice died by 80 days. The group treated with IL-2 only had a tumor with an average volume that was about 22% as that of the control group on the fifteenth day of the administration of the magnetite. In the group with IL-2 treatment only, one mouse died and 5 of the 7 surviving mice had uncontrolled growth. The CR for the group with IL-2 treatment only was 12.5%. The group with hyperthermia and IL-2 treatment had a tumor which was about 0.7% the volume as that of the control group after

15 days. Of the group treated with hyperthermia and IL-2 in combination, 6 out of 8 mice showed complete regression, although one had a relapse after the complete regression. Thus, CR was 75%. In Example 1, the control group, the group of hyperthermia treatment only, and the group of IL-2 treatment only all had a survival rate of 0% by 80 days. In contrast, the group with hyperthermia and IL-2 treatment combined had a survival rate of 62.5% by 80 days.

Example 2 of above-identified application: Treatment included a control group, hyperthermia only treatment, GM-CSF only treatment, and a combination of hyperthermia and GM-CSF treatment. In the control group, the group treated with hyperthermia only, and the group treated with GM-CSF only, seven of ten mice died by the 30th day and all the mice died by the 50th day. The group treated with hyperthermia and GM-CSF had a 50% survival rate after 50 days. In the group treated with GM-CSF only, CR was 0% since all the mice had increasing tumor size. In the group that had treatment with hyperthermia and GM-CSF, the CR was 40% since the tumor disappeared in four of the ten mice and no regression of the tumor was observed.

The information above demonstrates synergism between hyperthermia (one irradiation) and IL-2 or GM-CSF treatment regarding tumor size, CR, and survival rate. A summary of the CR, for example, is as follows:
Example 1: The CR for hyperthermia treatment alone plus the CR for IL-2 treatment alone was 12.5%. The CR for the combination of hyperthermia and IL-2 treatment was 75%, which shows a synergistic effect since the results were several times better.
Example 2: The CR for hyperthermia treatment alone plus the CR for GM-CSF treatment alone was 0%. The CR for the combination of hyperthermia and GM-CSF treatment was 40%, which shows a synergistic effect.
Cited references 1 and 2: The CR for hyperthermia treatment alone plus the CR for the IL-2 and GM-CSF

treatment was 35%. This is less than either the CR for the hyperthermia and IL-2 combination or the CR for the hyperthermia and GM-CSF combination. Thus, synergistic results have been demonstrated. Furthermore, it should be noted that cited document 2 used a combination of IL-2 and GM-CSF, which would provide better results than IL-2 and GM-CSF separately, so the synergistic effect is even greater than calculated above.

I believe that the above findings are surprising and unexpected from the test results of hyperthermia on the one hand, and the test results of IL-2 and/or GM-CSF on the other.

I further declare that all statements made herein of may own knowledge are true and that all statements made in information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: March 29, 2006

Takeshi Kobayashi

Takeshi KOBAYASHI